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Antiinflammatory Drug Effects on Ultraviolet Light-Induced Epidermal Ornithine Decarboxylase and DNA Synthesis

NICHOLAS J. LOWE, M.D., M.R.C.P., AND JAMES BREEDING, B.S.

Division of Dermatology, University of California, Los Angeles, California, U.S.A.

Ornithine decarboxylase which forms putrescine by the decarboxyalation of ornithine, is the first and probably the rate-limiting enzyme in the biosynthesis of the other polyamines, spermidine and spermine. Epidermal ornithine decarboxylase activity is greatly elevated in response to tumor promoting agents and ultraviolet light. The purpose of this paper is to report modification of ultraviolet-induced epidermal ornithine decarboxylase activity by antiinflammatory agents.

Topical triamcinolone acetonide and indomethacin were found to significantly inhibit the UV-B induction of epidermal ornithine decarboxylase in hairless mice when applied following ultraviolet light irradiation. The corticosteroid also showed inhibition of ultraviolet light increased epidermal DNA synthesis. Indomethacin failed to show any inhibition of DNA synthesis.

It is suggested that these assays may be used to study drugs that may modulate some ultraviolet light effects on the epidermis.

The activities of ornithine decarboxylase (ODC) and the levels of its biosynthetic product, putrescine, are elevated in various hyperproliferative cell systems. Furthermore, recent studies have shown that in adult mouse epidermis very high levels of ODC activity may be obtained by application of certain phorbol esters [1,2]. It has also been shown that irradiation of adult hairless mouse skin with ultraviolet light (mainly 290–320 nm, UV-B) will produce similarly high levels of ODC induction[3,4]. Epidermal macromolecular synthesis increases 48 hr following UV-B [5]. The induction of ODC occurred much earlier after UV-B than the subsequent increase in DNA synthesis and, in fact, occurred while DNA synthesis was still suppressed [3].

A smaller increase of epidermal ODC activity has also been shown to be present in the epidermis of patients suffering from psoriasis, and the same group of investigators showed that the topical application of a glucocorticoid inhibited this elevated polyamine biosynthetic enzyme activity [6].

The purpose of these investigations, therefore, was to assess the effects of different antiinflammatory agents on UV-B-induced epidermal ODC activity and DNA synthesis.

MATERIALS AND METHODS

2–3 month-old female hairless mice (Uscd strain) born within 7 days were randomized and housed 5 mice per cage for 1 week prior to experimentation.

The UV-B source consisted of 6 FS40 Westinghouse sunlamp tubes (peak emission at 313 nm). The UV-B output in W/cm^2 of this unit was measured using an IL-700 radiometer and an IL SEE 240 sensor fitted with a UV-B filter sensitive to radiation between 240 and 325 nm. It was connected to an IL-720 photodosimeter with a feedback circuit to turn off the lamps at the predetermined dose.

For irradiation mice were placed in a specially constructed 23 × 25 cm wire mesh and metal cage containing 24, 4 × 6 cm compartments, and the sunlamps were routinely used at a distance of 13 cm. The output of the sunlamps was measured at this distance. Care was taken to only use the center of the sunlamp to ensure uniform (± 5% range variation maximum) UV-B energy exposure.

Triamcinolone acetonide and indomethacin was each dissolved in a vehicle containing 30% propylene glycol, 70% isopropyl alcohol and used in the concentration shown in Tables I and II. 20 μl of these drugs or vehicle alone were applied to the back skin immediately following UV-B irradiation.

Assay of Ornithine Decarboxylase Activity

24 hr after treatment, mice were killed by cervical dislocation and the epidermis from individual mice was separated by brief heat treatment (55° for 30 sec). Epidermal ODC activity was determined by measuring the release of $^{14}CO_2$ from DL-(1-^{14}C) ornithine hydrochloride (specific activity, 49.9 mCi/mmole), obtained from New England Nuclear, Boston, Mass., as previously detailed [3]. The animals were sacrificed after 24 hr because this is near to the peak induction time of epidermal ODC activity following this amount of UV-B [3].

Tritiated Thymidine Incorporation into Epidermal DNA

The animals were injected intraperitoneally with 25 μCi of tritiated thymidine (Amersham/Searle: specific activity 15.7 μCi/mM). The animals were killed 1 hr later by cervical dislocation and skin specimens obtained for DNA extraction. The mice were sacrificed 48 hr after UV-B because this was the time of maximum increase in epidermal DNA synthesis after this amount of UV-B [3].

The hydroxyapatite column extraction of epidermal DNA followed the procedure described by du Vivier et al [7]. The epidermis was removed as for the ODC assay. It was homogenized by a Brinkman PT10 homogenizer. The results of epidermal DNA synthesis were expressed as counts per minute per μg DNA.

Statistical Analyses

Experimental group means were compared for significance by Student's *t*-test.

RESULTS

As summarized in Table I, topical administration of 0.01-0.1% triamcinolone acetonide resulted in a dose dependent ($p < 0.005$) inhibition of UV-B induced ODC activity. Indomethacin (2.5%) was also effective, although to a lesser extent.

The effects of these antiinflammatory drugs on UV-B induced DNA synthesis is shown in Table II. Triamcinolone acetonide (0.1%) significantly ($p < 0.005$) inhibited UV-B induced epidermal DNA synthesis. 2.5% indomethacin did not significantly alter the increase in DNA synthesis following UV-B treatment.

This concentration of indomethacin (2.5%) was chosen for the preliminary studies because it has been shown to be an optimum concentration in this vehicle for suppression of UV-B-induced skin erythema [15]. It is planned to conduct further experiments to establish a dose-response relationship for indomethacin.

DISCUSSION

Exposure to UV-B radiation results in epidermal cell death, an increased mitotic index, and an acceleration of macromolecular synthesis [3]. It has been recently shown that exposure of

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Reprint requests to: Dr. Nicholas J. Lowe, Division of Dermatology, University of California, Los Angeles, CA 90024.

Abbreviations:
ODC: ornithine decarboxylase

TABLE I. *Effects of triamcinolone acetonide (TMC) and indomethacin on UV-B induced epidermal ODC*

Experimental group[a]	# per group	Mean epidermal ODC (nmols CO_2/30 min/mg protein ± SD	% inhib	*p*-value rel to UV-B and vehicle[b]
UV-B + vehicle	4	1.69 ± .36	0	—
UV-B + 0.1% TMC	9	0.43 ± 19	75	$p < 0.005$
UV-B + 0.05% TMC	4	0.70 ± .20	58	$p < 0.005$
UV-B + 0.01% TMC	4	0.97 ± .11	43	$p < 0.005$
UV-B + vehicle	8	1.26 ± .44	0	—
UV-B + 2.5% indomethacin	8	0.59 ± .21	53	$p < 0.005$
Non UV-B irradiated control	12	0.046 ± 0.029	—	—

[a] The mice were treated with the drugs or vehicle immediately following UV-B exposure (0.08 J/cm^2). The mice were sacrificed 24 hr later.

[b] *p*-values obtained using Student's *t*-test.

TABLE II. *Effects of triamcinolone acetonide, indomethacin on UV-B-induced DNA synthesis*

Experimental group[a]	# per group	Mean epidermal DNA synthesis (cpm/DNA) ± SD	% inhib	*p*-value rel to UV-B + vehicle[b]
UV-B + vehicle	5	203 ± 47	0	—
UV-B + triamcinolone acetonide 0.1%	5	50 ± 19	75	$p < 0.005$
Non-irradiated control	5	87 ± 29	—	$p < 0.005$
UV-B + vehicle	4	417 ± 94	0	—
UV-B + indomethacin 2.5%	4	346 ± 97	17	$p < 0.2$
Non-irradiated control	4	154 ± 54	—	$p < 0.01$

[a] Mice were treated with the drugs or vehicle immediately following UV-B exposure (0.08 J/cm^2). The mice were sacrificed 48 h later.

[b] *p*-values were obtained using Student's *t*-test.

hairless mice to UV-B irradiation leads to a marked increase in epidermal ODC activity [3,4]. Significant induction of epidermal ODC occurred within 2 to 4 hr after UV-B irradiation and reached a peak at 24 to 28 hr before declining to normal. This increased ODC activity occurred, therefore, at a time when epidermal DNA synthesis remained depressed following UV-B and before the epidermal proliferative activity occurred. It has also been previously shown that UV-B induced ODC activity is depressed by treatment with cychloheximide, an inhibitor of protein synthesis, and by 5-azacytidine, an inhibitor of nucleic acid synthesis [4]. Du Vivier et al described an assay for antiproliferative drugs using principally 254 nm UV-C radiation [7]. They found that certain antiinflammatory corticosteroids, as well as antiproliferative drugs, were active in their model and were able to inhibit post-UV epidermal DNA synthesis. Our results seem to parallel these findings using, however, a different source (UV-B) for increasing DNA synthesis.

The mechanisms of inhibition of UV-B induced epidermal ODC by a topical steroid must remain speculative. ODC is thought to be specifically induced during the late G1 phase of the cell cycle [8]. Interestingly, it has also been shown that corticosteroids are able to block epidermal cells during both the G1 and G2 phases of the cell cycle [9]. The ability of the corticosteroids to inhibit UV-B induced ODC activity and DNA synthesis, therefore, again points to the ability of these drugs to act at several different points in the cell cycle.

Corticosteroids have also been shown to reduce the elevated epidermal polyamine biosynthesis in psoriasis [6] and have been shown to profoundly depress ODC enzyme activity in thymus and other lymphatic tissues [10].

There has been a report, however, that antiinflammatory corticosteroids did not inhibit phorbol ester-induced ODC activity in the skin of mice [11]. These results, which appear to be in conflict with the ability of corticosteroid to inhibit UV-B induced ODC activity, suggest that ultraviolet light and phorbol esters induce epidermal ODC by different mechanisms. The time course of response of induction of epidermal ODC is different after TPA phorbol ester induction [1] than after UV-B induction [3]. It is intended to conduct further time-dose experiments with different corticosteroids to examine the possibility of time-frame shifts of ODC response, as well as magnitude of ODC induction. Conversely, corticosteroids greatly stimulate ODC and S-adenosyl methionine decarboxylase activity in parenchymal organs, such as the liver [12].

It therefore seems likely that there are different mechanisms of action of these drugs on ODC activity in different tissues and in response to different stimuli.

It is interesting that indomethacin was capable of inhibiting UV-B induced epidermal ODC 24 hr after UV-B. It has been shown previously that the same concentration of indomethacin was able to suppress UV-B-induced skin erythema for the 24 hr following UV-B; however, it had no modulating effect on DNA synthesis changes seen during 48 hours following UV-B [13]. Indomethacin in our studies failed to s how any significant change in DNA synthesis following UV-B.

Greaves and Sondergaard first reported prostaglandin-like activity in perfusates from human skin that had been irradiated with ultraviolet light [14]. One pharmacological effect of indomethacin is prostaglandin synthetase inhibition and studies have shown that topical indomethacin at this concentration (2.5%) reduces sunburn erythema and prostaglandin E levels in sunburned guinea pig skin [15]. It has also been shown that it is possible to inhibit TPA-induced epidermal ODC activity by treatment with topical indomethacin [16]. There may be, however, several other possible mechanisms for the apparent epidermal ODC modulation by indomethacin other than postaglandin synthetase inhibition.

In summary, we have investigated the topical effects of certain antiinflammatory drugs on UV-B induction of the polyamine biosynthetic enzyme ODC. A corticosteroid and indomethacin were able to inhibit UV-B induced ODC activity. It remains to be seen whether these assays can be usefully employed as a means of evaluating antiproliferative, antiinflammatory and, perhaps, photoprotectant properties of different drugs.

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